Spawning Season and Sex Ratio of Echinoids

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During the past three years, various embryological projects in this laboratory have required the use of large numbers of gametes from large numbers of sea urchins, *Lytechinus variegatus* (Lamarck), and sand dollars *Mellita quinquiesperforata* (Leske). In order to preserve valuable data, records were kept on the number of these animals collected, and treated to induce spawning, since May, 1964. These records are a by-product of the other work, and thus do not include the precise locations of the collecting sites, the temperature of these locations, the size of all individuals collected, or histological preparations of the gonads. However, in view of the large number of animals involved (thousands), and since accurate temperature data are available for the Cedar Keys area from Coast and Geodetic Survey records, it was decided that the information should be made available for those wishing to work on these animals in Florida, either from the standpoint of their life history or their embryology. The data also have a bearing on the problem of sex determination in echinoids.

Methods

The animals were collected by random dredging in the channels or on the shallow banks in the vicinity of the University of Florida Marine Laboratory at Seahorse Key. Animals with test diameters smaller than two inches (*Lytechinus*) or two and one-half inches (*Mellita*)were discarded. The animals were kept in fresh sea water (changing several times) on the collecting boat, and were transported to Gainesville "dry" (in a bucket under a layer of paper towels soaked in sea water). The trip to Gainesville requires about one hour. Upon arrival, the animals were transferred to two-gallon sea water aquaria, and kept in a beverage cooler at 17 C. The aquaria were supplied with a constant flow of washed, compressed air. The animals remain viable for about two weeks under these conditions. No attempt was made to feed the animals, or to provide continued filtration of the aquaria. Spawning was induced by the injection of 1-5 ml of 0.55 M KCl (isotonic) into the coelom through the peristomial membrane

(Palmer, 1937; Tyler, 1949). "Ripe" animals shed gametes within 5 minutes. The sex of the animal is readily apparent on shedding in either *Mellita* or *Lytechinus*. Other sex limited characteristics are apparently lacking in both animals, though *Lytechinus pictus* (Verrill) and *Lytechinus anamesus* Clark from Southern California show sexual dimorphism of the gonopores, those of the female being statistically larger than those of the male (Tyler, 1944). No information is available regarding gonopore size of *L. variegatus*. The gametes were examined with phase contrast optics, and the presence or absence of fertilized eggs, sperm motility, primary oocytes, and eggless-jelly (*Mellita*) noted. The syringe used for KCl injection as well as the animals to be injected were rinsed with tap water to inactivate "stray" sperm. Such precautions, along with gross observation of spawning gonopores, allowed for diagnosis of hermaphroditism (none was noted).

Monthly spawning and temperature data for each year have been lumped into a three year total since no gross variation in these figures was evident within a given month. The average monthly temperature was taken as the average of the two diurnal extremes recorded for that particular month at Cedar Keys, Florida. The sampling frequency varied with the month and year between no collections and one collection every week. Each sample was assayed in its entirety for sex ratio and unresponsive individuals. The accumulative (three year) monthly sample sizes ranged between extremes of 16-264 individuals. The average sample size was 125 for both *Lytechinus* and *Mellita*. No collections of *Mellita* were made during July and August, and only a small August sample (16 animals) of *Lytechinus* is available. Totals for the entire three year period are tabulated (Table 1) and the proportion spawning (per cent) is presented graphically along with a plot of the temperature (Fig. 1).

TABLE 1
Spawning data for *Mellita quinquiesperforata* and *Lytechinus variegatus* from May, 1964, to May 1967

	Number	Male	Female	Unresponsive
Lytechinus	1504	317	205	952
Mellita	1121	381	294	446

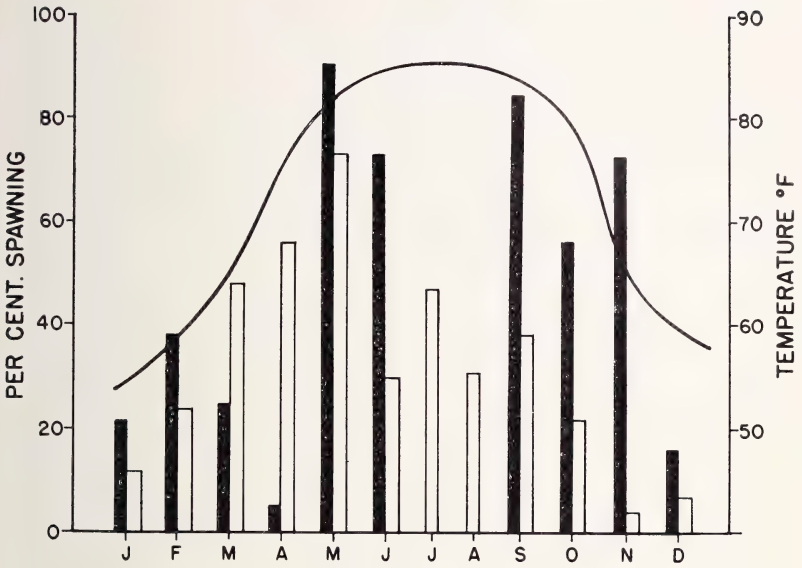

Fig. 1. Annual spawning cycle and local temperature. Black bars, *Mellita quinquiesperforata*. Open bars, *Lytechinus variegatus*. Per cent of animals responding to injected KCl (see text), ordinate. Months of the year (1964 to 1967), abcissa. Black line, mean diurnal temperature at Cedar Keys, Florida 1964-67.

RESULTS

Aside from the data tabulated below, the following information was noted. 1) There is no relationship between sex and size, in either animal, above the size limits previously stated. 2) Males of both populations tend to spawn spontaneously during the early part of the shedding period. This usually occurs during the return trip following collection. Females also shed spontaneously on rare occasions. 3) Collections during the cooler months frequently yield a high proportion of males, above 70 per cent. At *no time* during the collection period included in this report did the number of spawning *Lytechinus* females equal or exceed the number of males. The sex ratio (males:females) drops from a value of 5:1 in February to about 1.4:1 in April (*Lytechinus*). The same holds true for *Mellita*, though the sex ratio is closer to unity in this animal (1.3:1 overall). 4) Primary oocytes appear, immediately

following (two weeks) the initial observations of spawning males. These are inter-mixed with fertilizable, mature ova in concentrations approaching 20 per cent. 5) During the decline of the breeding period, *Mellita* females release eggless-jellies, observable macroscopically since the jelly coat of these eggs contains red echinochrome granules. These jelly coats presumably represent mature eggs which have been completely or partially absorbed following the main spawning period. The same may hold true for *Lytechinus*, since cytolyzing eggs are occasionally seen toward the end of the spawning season, though the jelly surrounding the eggs is devoid of pigment and of the same refractive index as sea water, thus being invisible even with phase contrast optics.

It is also apparent that, during the period in question and with the sampling methods used, males outnumber females by an overall ratio of 3:2 (*Lytechinus*) and 4:3 (*Mellita*). The apparent discrepancy from a 1:1 ratio of sexes is discussed below. Maturation of gametes unquestionably commences with warm weather, as one would expect, and probably proceeds on a continuous basis throughout the summer months in both animals. Synchronous spawning (perhaps triggered by the females, as they approach ripeness) presumably occurs soon after the initial ripening period in April and May. March of 1967 was the earliest that *Lytechinus* was available in abundance in ripe condition.

Discussion

It seems clear from the evidence above, that here is a preponderance of males in both populations, particularly *Lytechinus*. The overall sex ratio may still be 1:1 if one could sex all animals collected, ripe or not. One is, however, examining the populations for the ratio of ripe males to ripe females. Since no relationship between size and sex seems to exist, the explanation for the excess of males observed in the population is probably not to be found in the phenomenon of protandrous hermaphroditism (e.g., Moore et al., 1963). Rather, it seems likely that the males mature first and spawn in response to the shedding of the females when the latter become ripe. Having spawned, the males regenerate mature gametes faster than the spent females. Thus, at a given time the population will consist of more ripe males than ripe females. At the height of the breeding season, when the animals are found in

aggregates (see Tennent, 1910 and Moore, et al., 1963), nearly all the animals respond to the treatment. The preponderance of males is still observed (Table 2). Since it has been shown (Moore et al.,

TABLE 2
Spawning data for *Mellita quinquiesperforata* and *Lytechinus variegatus* during selected periods of maximum ripeness (more than 70% spawning)

	Number	Male	Female	Unresponsive
Lytechinus				
April-May 1967	131	81	50	0
Mellita				
May 1966	235	112	99	24

1963) that mature animals come together in beds, it is not surprising to note the continued excess of males, since solitary (unripe) animals would be least likely to be collected by dredging in the more or less random manner described (hunting for the area yielding the most animals per trial). The extent to which immature animals aggregate in comparison to mature animals is as yet undetermined. Corollaries of this theory would be: 1) That most solitary *Lytechinus* (and, perhaps, Mellita) found during the breeding season would be females. 2) The initial trigger for synchronous spawning would, in the absence of artificial stimulation, be the spawning of the female(s). The above statements apply to *Mellita* as well as *Lytechinus* with the following reservations: 1) Bedding activity in sand dollars would seem to be less marked than in *Lytechinus*, since sand dollars seem, in the author's experience, to be found mainly in rather restricted areas (or beds) regardless of season. 2) Misclassification of females as unresponsive individuals is less frequent in *Mellita* due to the presence of echinochrome granules in the jelly surrounding even degenerating eggs, rendering them macroscopically visible. The latter condition tends to even the sex ratio during the declining weeks of the breeding season.

The cytology of sex determination in sea urchins and sand dollars is not completely clear, mainly due to the small size and considerable number of the chromosomes. Some genera (*Tripneustes*, for one) appear to have an "XO" (digametic male)

mechanism (Tennent, 1912). Others, including *Lytechinus*, may have a similar mechanism (Tennent, 1912). Shapiro (1935) reports the sex ratio of *Arbacia punctulata* at Woods Hole to be 1.03:1 in favor of females (2,358 animals), a statistic compatible with an XO mechanism. Sexing in this case was done by cutting the animals open around the circumference of the test and recording gonad color (ovaries are red) as well as presence of eggs or sperm. Hermaphrodites such as those described by Moore et al. (1963) and others (numerous reports exist, see Harvey, 1956; Hyman, 1955; Boolootian and Moore, 1959) should be investigated cytologically to determine whether or not there is a chromosomal basis for this condition, rather than a phenotypic explanation. Until further information is available, it seems unnecessary and unwarranted to assume anything different from a 1:1 sex ratio, in the total population, of either *Lytechinus* or *Mellita*. The assumption of a differential rate of gametogenesis, the males being more rapid than the females in the production of gametes, is sufficient to account for the observed departures from a 1:1 ratio in the breeding population.

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Literature Cited

Boolootian, R. A. and A. R. Moore. 1959. A case of ovotestes in the sea urchin *Strongylocentrotus purpuratus*. Science, vol. 129, pp. 271-272.

Harvey, E. B. 1956. The American Arbacia and other sea urchins. Princeton University Press, Princeton, N. J., 298 pp.

Hyman, L. H. 1955. The Invertebrates. Vol. 4. Echinodermata. McGraw-Hill, New York, 746 pp.

Moore, H. B., T. Jutare, J. C. Bauer, and J. A. Jones. 1963. The biology of *Lytechinus variegatus*. Bull. Mar. Sci. Gulf and Carib., vol. 13, pp. 23-53.

Palmer, L. 1937. The shedding reaction in *Arbacia punctulata*. Physiol. Zool., vol. 10, pp. 352-357.

Shapiro, H. 1935. A case of functional hermaphroditism in the sea urchin, *Arbacia punctulata*, and an estimate of the sex ratio. Amer. Nat., vol. 69, pp. 286-288.

TENNENT, D. H. 1910. Variation in echinoid plutei. Jour. Exp. Zool., vol. 9, pp. 657-714.

TENNENT, D. H. 1912. The correlation between chromosomes and particular characters in hybrid Echinoid larvae. Amer. Nat., vol. 46, pp. 68-75.

TYLER, A. 1944. Sexual dimorphism in sea urchins. Anat. Rec., vol. 89, p. 573.

———. 1949. A simple, non-injurious method for inducing repeated spawning of sea urchins and sand dollars. The Collecting Net, vol. 19, pp. 19-20.

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